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Note: The pictures of the virus particles on the cover are electron micrographs taken at Plum Island. They are magnified c. 175,000 times.

This publication supersedes Miscellaneous Publication 931, "The Plum Island Animal Disease Laboratory."

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THE PLUM ISLAND ANIMAL DISEASE CENTER

Research on Foreign Diseases of Animals

The Plum Island Animal Disease Center is the only research facility in the United States devoted to the study of contagious foreign diseases of animals. It is located on an island east of Long Island, N.Y., and is operated by the Agricultural Research Service, U.S. Department of Agriculture.[1]

The Department is responsible for:

- Developing capability of diagnosis of animal diseases that do not exist in the United States.
- Conducting basic and applied research on foreign animal diseases and their causative organisms.
- Developing adequate procedures so that foreign, domesticated, and wild animals, and semen, meat, and other animal products may be imported safely.

The Center conducts fundamental research to develop the necessary knowledge that enables the Department to carry out these responsibilities. The main objective is to prevent the introduction of diseases that could result in high death tolls or serious economic losses in our susceptible livestock population.

LOCATION AND HISTORY

Plum Island is located 110 miles from New York City, about 10 miles from Connecticut, and about 1-1/4 miles off the northeastern end of Long Island, N.Y. The island contains about 800 acres and is about 3 miles long and a mile wide in the western, or widest part. It is reached by boats operated by the Center from Orient Point, Long Island, where the Center has a harbor, an office building, and storage facilities for incoming supplies.

Plum Island was named by early explorers who observed beach plums growing along the shores. In 1659 the ruling Indian chief of Long Island sold Plum Island to the first European owner, Samuel Wyllis, for "a coat, a barrel of biscuits, and 100 muxes[2] or fishhooks."

The U.S. Government bought the island in the 1890's and established Fort Terry, a coast artillery post. The island was assigned to the Army Chemical Corps after World War II. On July 1, 1954, all of Plum Island, except for a U.S. Coast Guard lighthouse, was formally transferred to the U.S. Department of Agriculture for the establishment of a Center for the study of exotic diseases of domestic animals.

Preliminary studies were started in 1954. When additional laboratory facilities became available in 1956 the Center's research was expanded into a broad program covering many foreign animal diseases.

THE CENTER'S MISSION

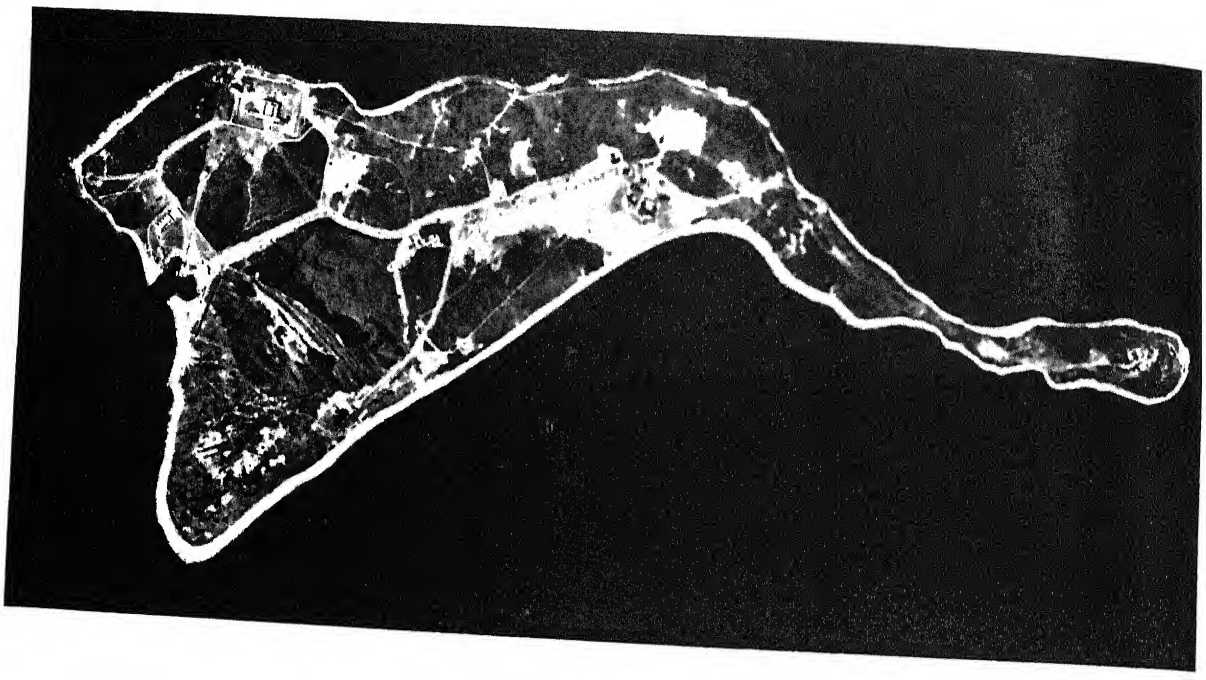
The mission of the Center is to perform the following basic and applied *research* and *service* work on the various contagious foreign diseases of animals, with primary emphasis on foot-and-mouth disease.

Research

- Basic research on viral structure, pathogenesis of the disease, antigen-antibody

[1] Mailing address: P.O. Box 848, Greenport, Long Island, N.Y. 11944.

[2] Muxes are small drills the Indians used to make holes in wampum.



Aerial view of Plum Island. PN-3648

reactions, and host and disease-agent relationships.

- Applied research on virus survival in animals and animal products, methods of virus inactivation, and development of vaccines and other control measures.

Service

- Diagnosis of disease by laboratory tests on specimens from animals in suspected field outbreaks.
- Tests for infectious agents in semen or specimens from live animals prior to importation.
- Assessment of hazards from imported products.
- Production of diagnostic materials for other laboratories.
- Training of U.S. and foreign personnel.
- Technical assistance to foreign countries to lower disease rates and thus reduce hazards to the United States.

Technical support to other Federal agencies includes diagnostic services, specialized studies on animal products, and development and evaluation of new techniques.

Emergency services are performed as required for diagnosing foreign animal diseases. When materials from disease outbreaks of suspected foreign origin are submitted to control agencies, studies are conducted to determine whether a foreign animal disease is involved.

Tests are made on throat fluids, serum, and semen to determine whether animals or semen for importation may be infected with foot-and-mouth disease virus.

Training courses are given so that diagnosticians in the field will become more familiar with the foreign diseases of animals, which they may have to recognize and investigate if outbreaks occur.

Specialized studies on animal products such as meat and semen are made to assist control agencies in deciding whether certain animal products should be admitted from foreign countries and what may be done to render them safe from a disease standpoint. Certain foreign biological products require a similar safety evaluation. If this service had been available in 1908, an outbreak of foot-and-mouth disease in this country might have been averted. The outbreak was traced to contaminated imported smallpox vaccine, which was propagated in calves.

New disinfectants and sterilization techniques also are evaluated to assist the work of control agencies in dealing with foreign animal diseases.

SAFETY PRECAUTIONS

Because of the Center's location as well as its special facilities, devastating foreign animal diseases can be studied without endangering livestock on the mainland. Congress provided this protection for U.S. livestock by specifying that the Center be on an island entirely under Federal control and be separated from the mainland by deep navigable water.

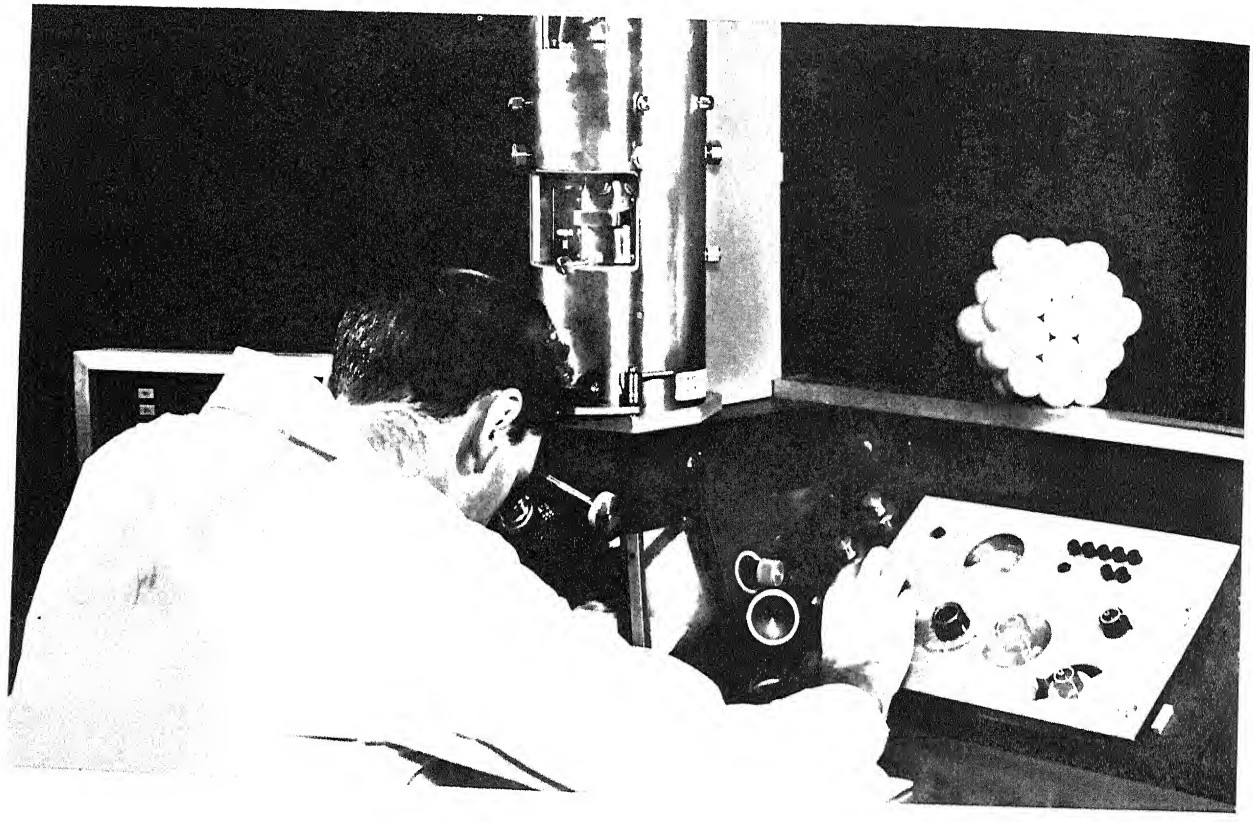
Rigid safety regulations were also devised to prevent the escape of highly communicable disease- causing agents from one research area to another and the accidental introduction of extraneous domestic disease agents, which would complicate the studies.

The Federal Government controls all movement to, from, and on the island. Only authorized persons are permitted entry to the island; entrance to the laboratory buildings and animal quarantine areas is restricted. All Center personnel are prohibited from contact with susceptible species of animals, or premises where such animals are held, for specified periods of time after leaving the island (7 days for persons working in the laboratories and 3 days for other personnel).

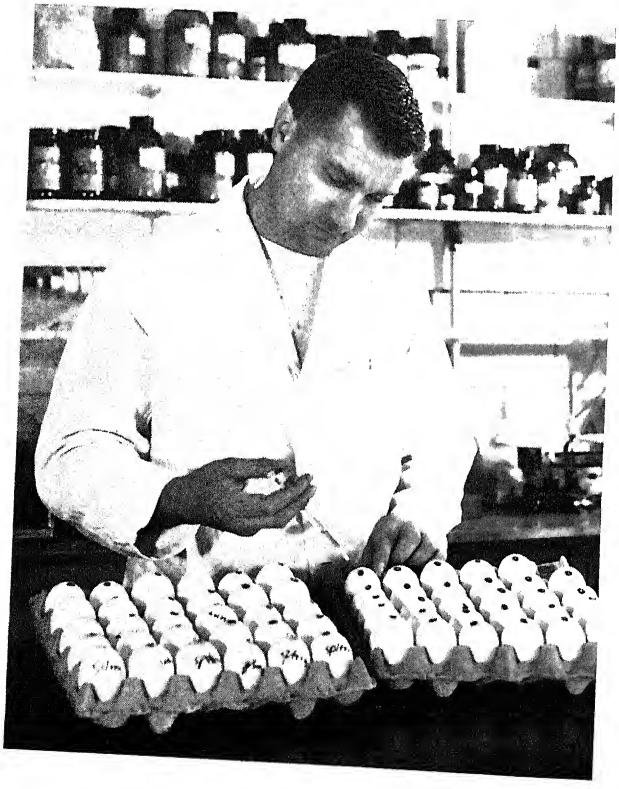
The two main laboratory buildings on Plum Island were specifically designed for research on highly communicable diseases and are considered among the safest in the world for work on animal viruses. All entrances and exits

PN-3649

The "M. S. Shahan" is used to transport employees to and from Plum Island.



A scientist uses the electron microscope. PN-3650

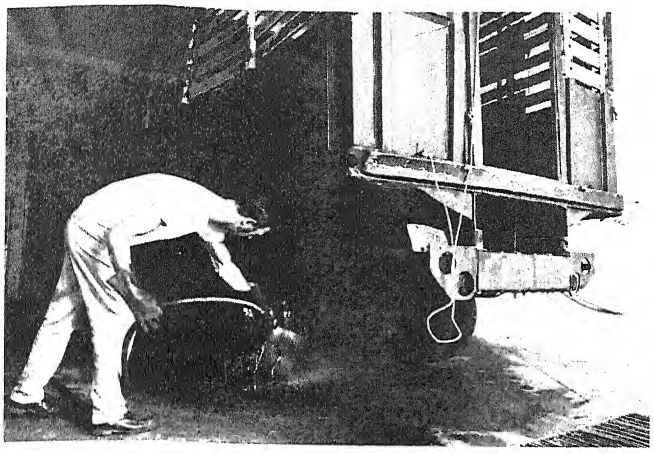


PN-3651
A technician inoculates chicken egg embryos to detect virus.

for personnel, animals, and supplies are strictly controlled. Persons must change to laboratory clothing upon entering the building. Upon leaving, they must take a decontaminating shower before putting on their own clothing.

Exhaust air from these buildings is decontaminated through a system of filters, and all liquid

PN-3652
Foreign scientists observe a rapid diagnostic test for African swine fever.



PN-3653
A technician disinfects a truck that must be removed from Plum Island.

PN-3655
Genetically stable laboratory mice are raised especially for Plum Island research.

wastes are sterilized by heat before being discharged. Solid wastes, including animal carcasses, are destroyed by incineration within the research buildings.

SUPPORT ACTIVITIES

Because of its isolation, the Center maintains all services needed to support its research. It employs an administrative staff, engineers, animal caretakers, maintenance men, a safety staff, guards, firemen, and other workers, in addition to scientists and laboratory technicians.

PN-3654
Cattle are introduced into the laboratory through air locks.

The Office of the Director provides overall guidance and management for the research and supporting groups.

Administrative Management Services is responsible for personnel work, purchase and delivery of supplies, and operation of food, photographic, and duplicating services.

The Safety Office places major emphasis on preventing the escape of disease agents from the Center. Other programs include industrial and fire safety, first aid, and plant security.

Animal Supply maintains colonies of disease-free guinea pigs, mice, and other small laboratory species to supply research sections. All large experimental animals, such as cattle, sheep, goats, and swine, brought to the island are inspected. Quarantines are placed on all animals until they are needed for research. Animal Supply also provides whole blood, tissues, and serum from normal animals for use in diagnostic tests and tissue cultures.

Laboratory Services provide tissue cultures and prepare media and sterile equipment for use in the laboratory. This group also ope
laundry and a glassware-washin

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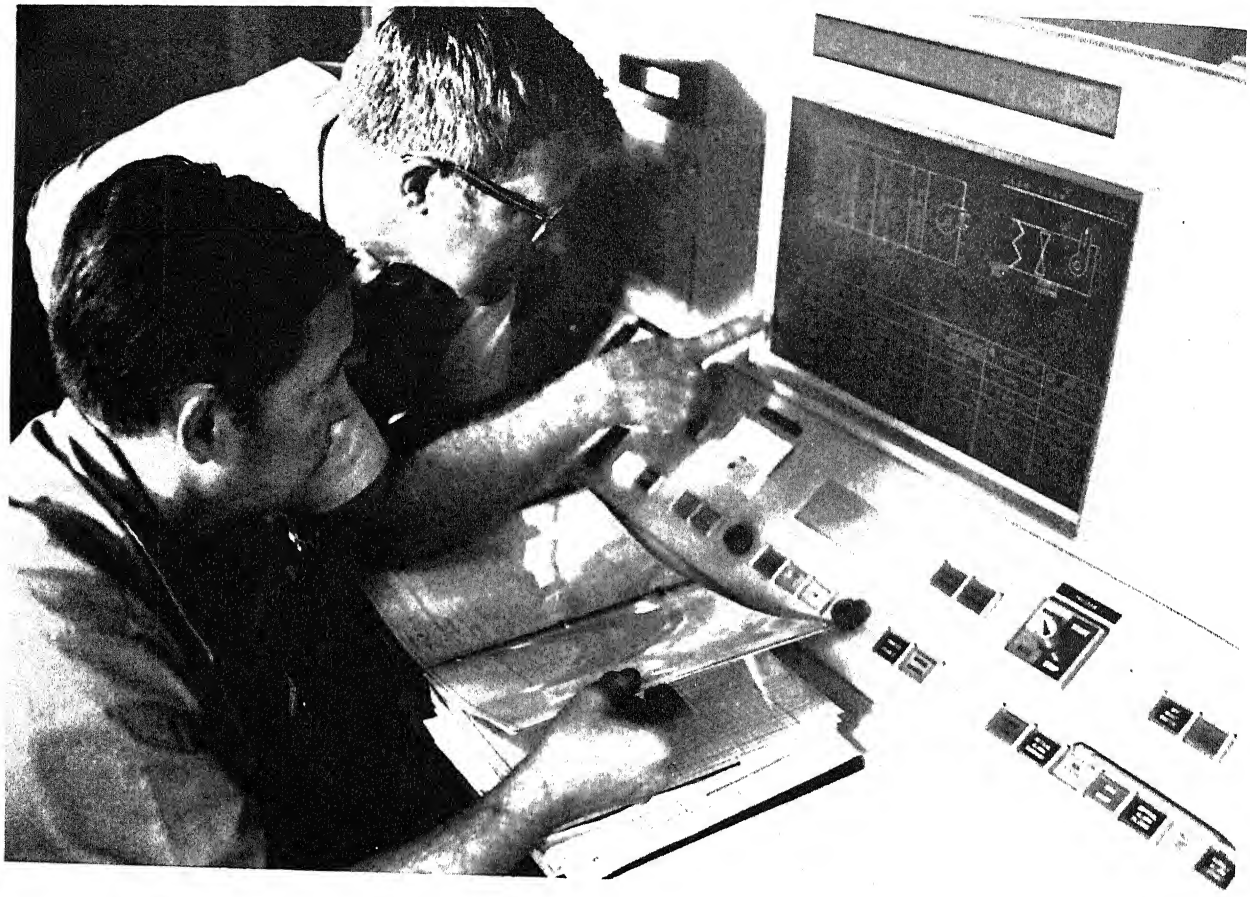


A supervisory data center is used to control and monitor equipment at Plum Island. PN-3656

support services such as electrical power, heating, water, sewage decontamination and processing plus marine and automotive transportation.

DISEASES STUDIED

The contagious foreign animal diseases studied and diagnosed in the Plum Island Center and the principal domestic animals they infect include—

- Foot-and-mouth disease—cattle, hogs, sheep, goats.
- Rinderpest—cattle.
- Teschen disease—hogs.
- African swine fever—hogs.
- Fowl plague—poultry.
- African horsesickness—horses, mules, asses.
- Asiatic Newcastle disease—poultry.
- Lumpy skin disease—cattle.
- Ephemeral fever—cattle.
- Duck virus enteritis—ducks.
- Vesicular exanthema of swine—swine.
- Louping ill—sheep.
- Ovine and caprine pox—sheep, goats.
- Nairobi sheep disease—sheep.
- Rift valley fever—sheep, cattle, goats.
- Bovine herpes mammillitis—cattle.
- Exotic vesicular stomatitis—cattle, sheep, goats, swine, horses.
- Swine vesicular disease—swine.
- Borna disease—horses.
- Peste des petits ruminants—sheep, goats.
- Equine encephalosis—horses.
- Contagious bovine pleuropneumonia—cattle.
- Contagious caprine pleuropneumonia—goats, sheep.
- Contagious agalactia—sheep, goats.
- East Coast fever—cattle.

The diseases listed are caused by viruses except for the last four. Contagious bovine and

caprine pleuropneumonias and contagious agalactia are caused by mycoplasmas and East Coast fever by a blood parasite (hematozoan). Some of the diseases affect wild animals and birds in addition to domestic animals. About 70 percent of the research and service work is devoted to foot-and-mouth disease because of its great economic importance. Techniques and materials are being developed for rapid diagnosis of this and the other foreign diseases in the event of outbreaks here.

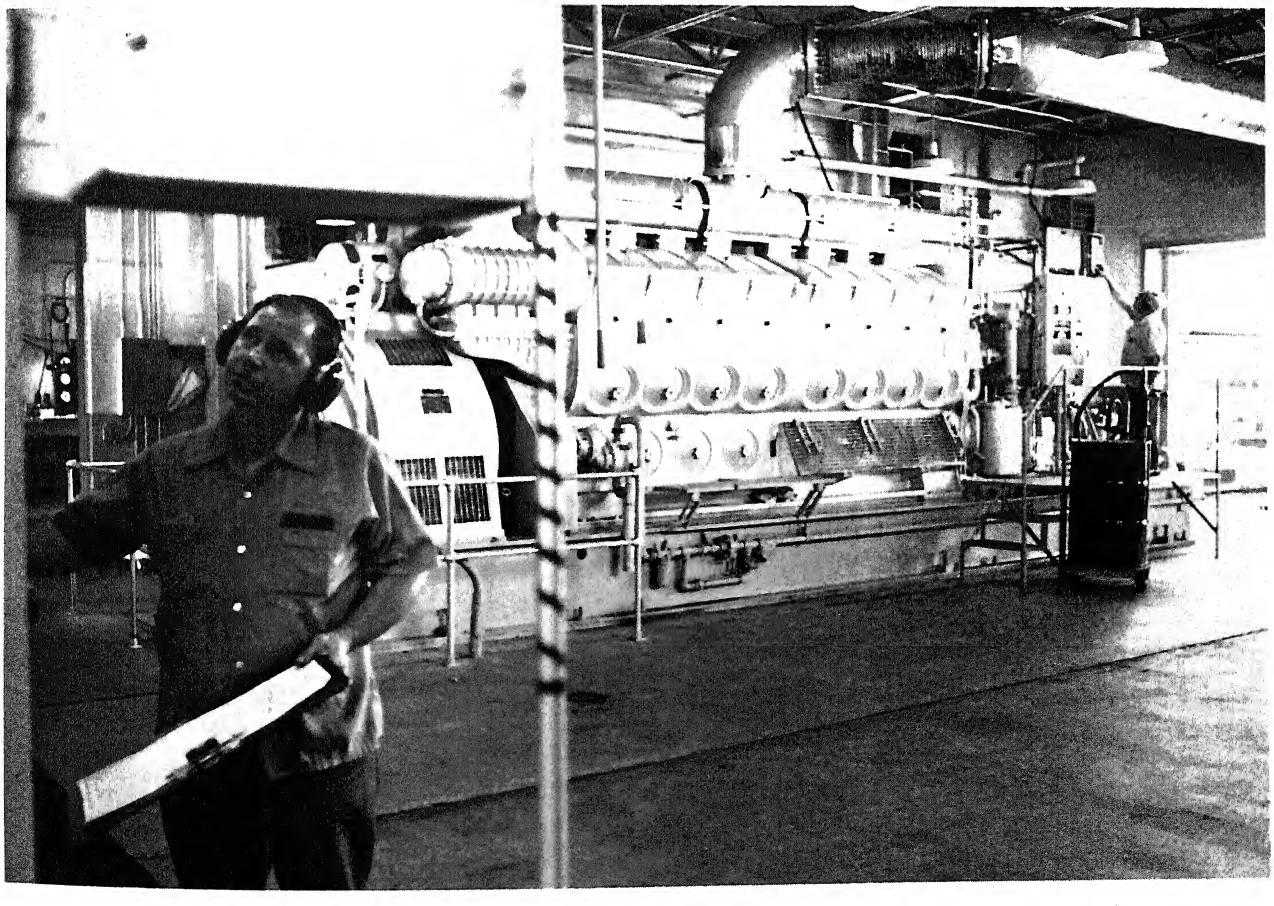
The Center's program is flexible enough to allow the study of additional disease problems when necessary. An outbreak of duck virus enteritis (duck plague), in the Long Island duck industry, necessitated a comprehensive study of this disease. When hog cholera is officially eradicated from this country, it also will be added to the list of foreign diseases to be studied and diagnosed.

GENERAL AREAS OF RESEARCH

Research at the Center has been divided into five general areas. These areas broadly correspond to the work of five different research groups, or disciplines. The general areas of research are as follows.

Viruses
(1) Biophysics
(2) Protein coat structure
(3) Nucleic acid structure
(4) Synthesis
(5) Antiviral agents

Vaccines
(1) Virus production
(2) Inactivants
(3) Adjuvants
(4) Safety and serologic testing
(5) Immunity challenge



PN-3657

Standby electric generators are always ready in case power from Long Island is cut off.

Cell Cultures

(1) Kinds of cells
(2) Cell nutrients
(3) Virus yield
(4) Viral changes
(5) Interference

Control Measures

(1) Virus persistence in animals, products, and environment
(2) Carrier studies
(3) Pathology and diagnosis
(4) Epizootiology and host range
(5) Disinfection

Diagnostic Tests

(1) Complement fixation
(2) Neutralization
(3) Agar gel diffusion
(4) Fluorescent antibody
(5) Agglutination

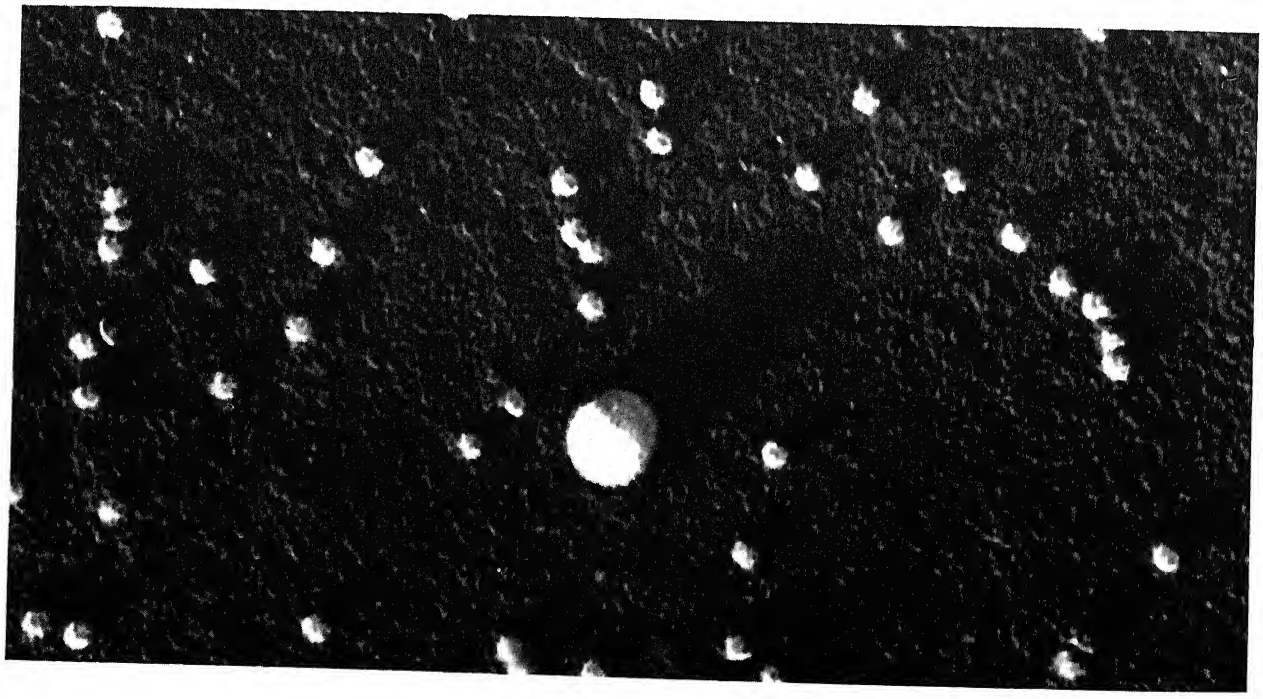
RESEARCH DISCIPLINES

The five research disciplines are biochemical and biophysical, immunological, cytological, microbiological, and diagnostic investigations. These research disciplines are composed of veterinarians, virologists, bacteriologists, pathologists, chemists, physicists, and their technical assistants. Working alone or as teams, the scientists and their assistants are assigned to one of the five research disciplines.

Biochemical and Physical

Scientists in biochemical and physical investigations are concerned with problems in molecular biology. These scientists produce milligram quantities of foot-and-mouth disease virus in cultures of baby hamster kidney cells and purify the virus for use in biochemical and immunological studies. They examine animal virus particles for their size and shape by electronmicroscopy and for their chemical properties, including resistance to mechanical treatment, pH changes, thermal changes, and variations in ionic strength. They determine the effects of enzymes and chemicals as purifying agents and inactivants; study viruses intact and broken down into their protein and infectious nucleic acid subunits; determine diffusion, electrophoretic, and sedimentation rates of viruses and their subparticles.

The scientists in this group also investigate the correlations of physiochemical properties with infectivity, immunogenicity, and antibody-



An electron micrograph shows foot-and-mouth disease virus particles. PN-3658

PN-3659

A technician operates a shadow casting instrument so that virus preparations can be viewed as three-dimensional objects.

antigen relationships. They study the mechanism of virus synthesis and its inhibition in tissue culture and cell-free systems by biological and chemical methods, using radiobiological tracers.

Immunological

Scientists in immunological investigations check the response of animals infected with or vaccinated against disease agents and they conduct research on the antibodies that protect against disease. Serum from these animals is separated into elemental components, and these are then analyzed by serological, chemical, and animal-testing techniques. They also study antigens or viruses that cause the disease.

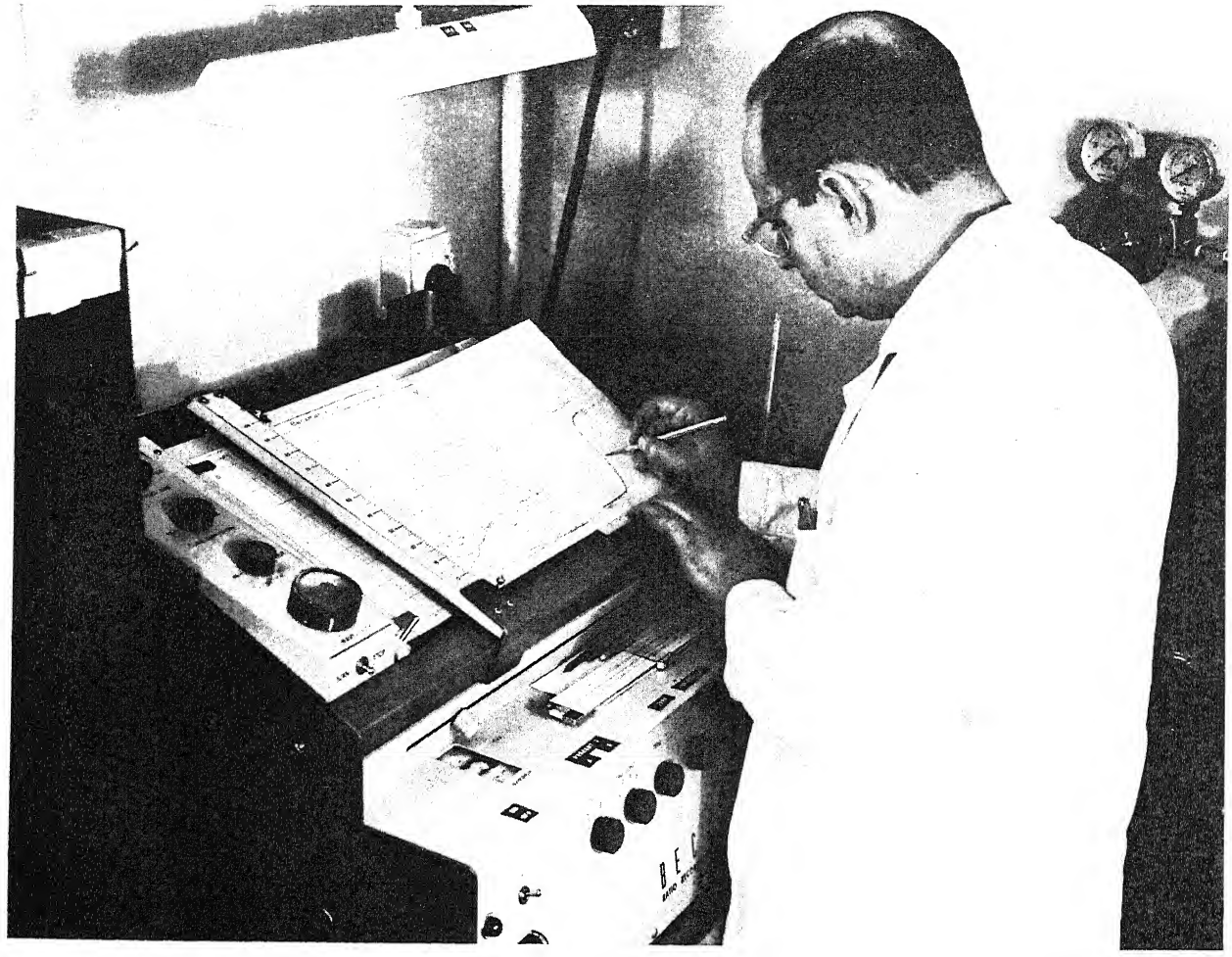
Another function of this group is the development and testing of vaccines appropriate for possible use if established disease-eradication procedures should fail to control invasions of foreign diseases. These studies include chemical inactivation of virus and development of critical tests to determine the safety and potency of vaccines produced. Such research requires the vaccination of many animals and the study of their immunity by serological and challenge methods.

Cytological

Scientists in cytological investigations study viral inhibitory substances, changes in viruses caused by environmental conditions, and growth of viruses in cell cultures. They have found that treatment of cells with certain chemicals stimulates the production of inhibitory substances effective to a limited extent against foot-and-mouth disease virus. They are investigating the possibility that these substances may be useful in the prevention of the disease.

Other scientists are attempting to change the foot-and-mouth disease virus by various procedures so that it no longer produces disease and thus might be used as a live attenuated virus vaccine.

Work in the cytological group also includes investigations on the viral susceptibility of various types of cell cultures and factors that



PN-3660

A scientist studies the results of foot-and-mouth disease virus on a ratio recording instrument.

affect their susceptibility. These studies are being done to obtain highly susceptible cultures for diagnosis of viral diseases and for other work involving assay or production of viruses.

Microbiological

Scientists in microbiological investigations study the susceptibility of various species of animals to virus diseases, explore ways in which the diseases spread, and determine in what organs and tissues the virus may be found. They study the factors that result in animals becoming virus carriers. They also trace the survival of viruses in meat, blood, semen, and other animal products. From the results of these studies, the U.S. Department of Agriculture is able to assess the hazards of importing live animals and animal materials from foreign countries in which dangerous diseases exist.

Scientists also study the effects of chemical and physical environments on viruses and thus contribute to knowledge regarding methods of virus inactivation, disinfection of contaminated materials and premises, and survival of viruses under various conditions. Such information is vital in preventing disease and eradicating outbreaks.

Diagnostic

When USDA veterinary diagnosticians in the field observe animals showing clinical signs suspicious of foreign animal disease, they collect samples and submit them to Plum Island. At

Plum Island, the staff of diagnostic investigations conducts various serological tests, virus isolations, animal inoculations, and pathological studies to determine if the samples were positive or negative for a foreign animal disease.

Considerable work is required to have in readiness all of the virus strains, antiserums, and cell cultures needed for the various diagnostic tests. In addition, research is conducted on various aspects of foreign diseases of animals other than foot-and-mouth disease.

RESEARCH HIGHLIGHTS

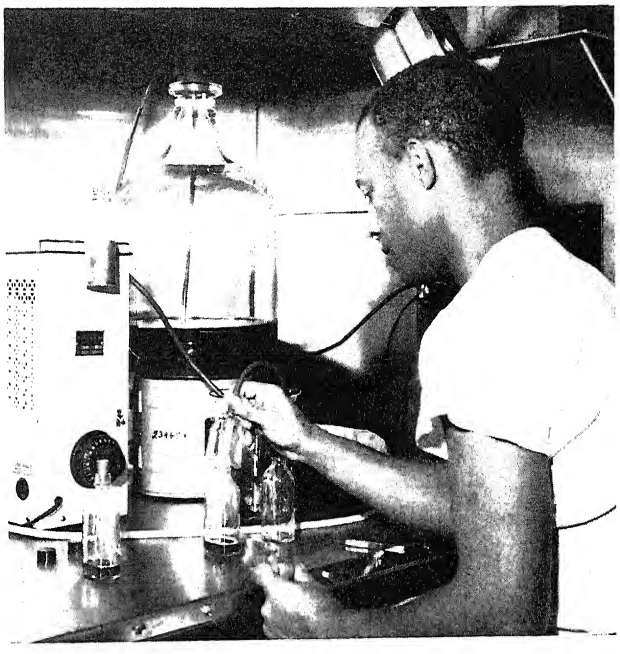
Foot-and-Mouth Disease (FMD)

Viewed foot-and-mouth disease virus (FMDV) in the electron microscope as a spherical particle 23 nanometers (about one-millionth inch) in diameter with 32 capsomeres on its surface.

Established that FMDV ribonucleic acid (RNA) can be encapsidated in a bovine enterovirus (BEV) protein coat and that such viral particles have biophysical properties of BEV but can produce FMDV in further growth cycles.

Developed an *in vitro* system for the study of the synthesis of FMDV-RNA.

Showed that FMDV-infected cells contain an enzyme, RNA polymerase, which is induced by the virus, is necessary for viral replication, but is not found in normal cells.



PN-3662

A technician dispenses kidney cells into tissue-culture bottles.

PN-3661

A technician cultures swine blood to determine sterility.

Showed that the virus replication activity of RNA polymerase is inhibited by antibodies produced in FMD-infected animals. Thus, RNA polymerase may be identical to a virus-infection-associated antigen (VIA).

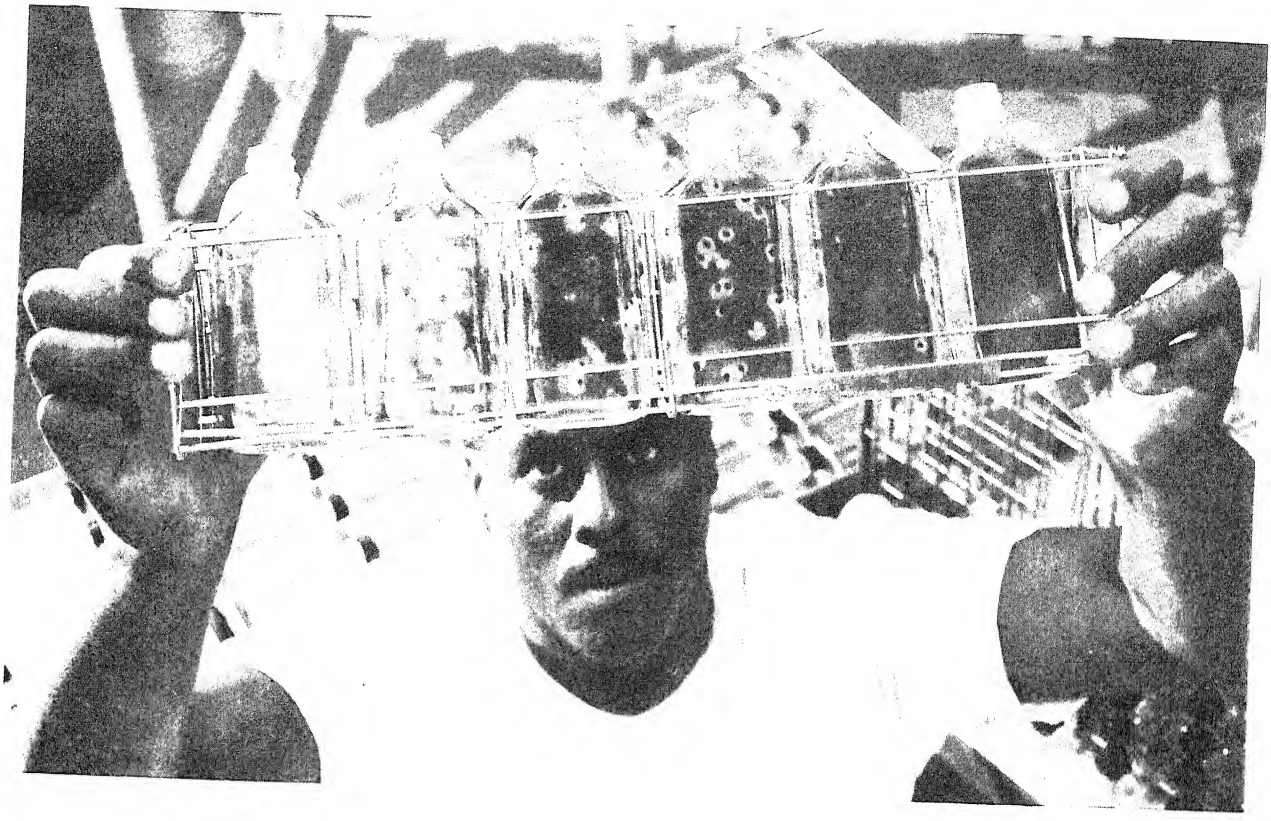
Demonstrated that VIA made from extracts of FMDV-infected cell cultures reacted with antibodies in serums from FMD-infected animals in agar gel precipitin tests, forming a band distinct from that of whole FMDV. A similar finding was demonstrated by means of fluorescent antibody techniques.

Established that the FMDV infection associated antigen used in the VIA agar gel diffusion test was a valuable tool for epizootiological surveys.

Found that FMDV persisted in cell cultures made from the pharyngeal and esophageal cells of infected cattle for as long as 24 weeks.

Found that FMDV can infect cattle, sheep, and goats and multiply in the upper respiratory tract regardless of their immune status, and that this infection and multiplication can occur in the complete absence of clinical signs.

Showed that a steer after intranasal inoculation with FMDV could transmit FMDV for 7 to



A technician shows plaques formed by virus particles found in the blood of steers. PN-3663

8 days and that the most infectious period was during the third day.

Found a latent form of FMD characterized by virus isolation from the blood in the absence of specific antibody development and with extremely long incubation periods.

Determined that FMDV may be present in semen of infected bulls before and after clinical signs of disease and that it may be transmitted to cows by artificial insemination.

Established that FMDV survives in lymph nodes and blood of beef carcasses for as long as 60 days, in bone marrow for more than 6 months, and in lymph nodes of wet, salt-cured meat for as long as 50 days.

Developed radial immunodiffusion procedures for measuring FMDV in crude tissue culture fluids as well as in concentrated and purified
rations.

ed that FMDV could be spread by air
ected to clean areas.

lished a facility for the large-scale culti-
f baby hamster kidney cells in rolling bottles and the production, therefrom, of 100 milligrams per week of purified FMDV.

Determined the dose of ionizing radiation required to inactivate FMDV and its RNA.

Found that FMDV is inactivated by organic acids, and by ethylene oxide gas, when sufficient humidity is present, and that beta-propiolactone, acetylethyleneimine, ethylene oxide may be used as inactivants when retention of antigenicity is desired.

Used polyethylene glycol for precipitation of FMDV for vaccine production.

Showed that a FMDV vaccine combining oil adjuvant can be used in a vaccination program involving revaccination, reducing the number of vaccinations per year and giving adequate protection for 6 months or longer.

Determined that purified FMD viruses inactivated and combined with oil adjuvants produced the first satisfactory vaccine for swine.

Showed the possible relationship between swine vesicular disease virus and Coxsackie B_5 virus of humans.

PN-3664

Plaques formed by foot-and-mouth disease virus particles in tissue culture cells.

Other Diseases

Isolated vesicular stomatitis virus from an infected human being.

Developed a rapid laboratory diagnostic test for African swine fever, in cooperation with the East African Veterinary Research Organization.

In cooporation with the same group, established methods for growing the schizont form of East Coast fever parasites in cell cultures. This technique modifies the parasite for potential use in a vaccine.

Developed, in cooperation with the East African Veterinary Research Organization, diagnostic tests for contagious bovine pleuropneumonia in formalized lung tissues using fluorescent antibody and agar gel diffusion techniques.

Purified the attenuated duck enteritis virus and developed a seed virus, which was supplied to the duck industry for production and use as a vaccine. This work was made possible by cooperation of Dutch scientists who supplied the starting materials.

THE CENTER'S FUTURE

As the world human population increases and the food supply becomes less abundant for each individual, the need to reduce losses from animal diseases becomes more important. The Center already has found and will continue to find new ways such as rapid diagnostic tests, control measures, and vaccines to limit or prevent outbreaks of foreign animal diseases.

Increased demand for food supplies involves developing faster growing and improved types of livestock, which in turn requires importation of animals and semen with the special genetic background to develop inbred and hybrid progeny of the desired type. Here again the Center is called upon to develop sensitive tests for detecting disease agents so that such importations may be made with a minimum of risk. Thus the Center has an important role in the development of future food supplies from livestock.

Basic research at the Center should continue to develop new techniques and concepts. However, applied research will receive more emphasis than it has in the past to put into service the improved techniques and findings that have been made through basic studies. Also, the results will have application to many other branches of medical science.

The very nature of research prevents the prediction of the exact character and the timing of conclusive results, but achievements of the program already have been outstanding. In the years ahead, the Plum Island Animal Disease Center undoubtedly will continue to add to the achievements of U.S. and international research.